REMARKS

I. Status of the Claims

Claims 1-35 are pending in the application. Claims 2, 28 and 31-34 stand withdrawn pursuant to a restriction requirement and are hereby canceled. Claim 30 is objected to, and claims 1, 3-27, 29, 30 and 35 stand rejected under 35 U.S.C. §112

II. Objection

Claim 30 is objected to, but the objection has been rendered moot in light of the cancellation of this claim.

III. Rejection Under 35 U.S.C. §112, First Paragraph

Claims 1, 3-27, 29, 30 and 35 stand rejected under the first paragraph of §112 as lacking enablement. Claims 6-8, 29, 30 and 35 have been canceled, leaving claims 1, 3-5, and 9-25 pending and under examination. The rejection is traversed as to the amended/remaining claims for the following reasons.

According to the examiner, the claims are not enabled for practicing of the invention as broadly claim, but instead, are supported only to the extent of protecting a mouse from an organophosphate comprising administering to the mouse an expression construct comprising a promoter linked to a PON1 gene, wherein expression of the PON1 results in detoxification of the organophosphate. While applicants traverse the rejection, the claims have been amended to recite protection (not treating) from organophosphate toxins (not all toxins). Thus, what appears to remain as the sole issue is whether applicants specification enables the treatment of cells and subjects other than mice.

A. Standard of Examination

According to MPEP §2164.04, the examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. Only if an examiner can provide reasons sufficient to create a reasonable doubt as to the accuracy of a particular broad statement put forward by applicant as enabling support for a claim, a rejection under 35 U.S.C. §112, first paragraph can be made. In other words, a specification which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented ***must*** be taken as in compliance with the enabling requirement of the first paragraph of §112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. *In re Marzocchi*, 169 USPQ 367 (CCPA 1971). With that framework in mind, applicants address the examiner's non-enablement position.

B. References Cited as Undercutting Enablement of Gene Therapy

The first argument advanced by the examiner is a general attack against gene therapy. A number of references are cited, including Anderson *et al.* (1998) and Verma *et al.* (1997). Applicants submit that these references, which actually summarize the state of the art ***in preceding years***, are all but irrelevant to the issue of enablement of gene therapy ***for an application claiming benefit of a filing date in 2001***. In this fast-moving field, a reference 3-4 years old is hopelessly outdated. The remaining references cited – Davis, Schmidt-Wolf *et al.* and Stribley *et al.* – are not discussed at all in the action, and thus applicants submit that the examiner has failed his burden in establishing unpredictability at the time of filing. Applicants

do note, however, that a cursory review of these latter three references supports enablement of the present invention:

> **Davis**[1]: "Non-viral delivery systems have the potential to create viable pharmaceuticals from nucleic acids .... *In vivo* problems ... are also encountered [but] ... [r]ecent progress ***has been made*** in overcoming these issues" (emphasis added). *See Abstract.*
>
> **Schmidt-Wolf *et al.*:** "Clinical gene therapy ***has been therapeutically beneficial*** in some patients with inherited disease, and it ***is expected that similar benefits will be produced*** in patients with other diseases, such as cancer ..." (emphasis added). *See Conclusions.*
>
> **Stribley *et al.*:** "Concerns about the safety of human gene therapy research are being actively addressed, and remarkable progress in improving DNA transfer ***has been made.*** The first treatment success for a genetic disease (severe combined immunodeficiency disease) has been achieved, and ongoing research efforts will eventually yield clinical applications in many spheres of reproductive medicine" (emphasis added). *See Abstract (Conclusions).*

Each of these papers actually ***supports*** the enablement of gene therapy, and does so ***at the time of filing.*** Thus, applicants submit that the "evidence" of record on the enablement of gene therapy general favors applicants position.

Applicants would further submit that nothing in this aspect of the rejection addresses the ***claimed*** invention. Rather, it seems primarily to cast dispersions on gene therapy as a general matter (indeed, the outdated Verma *et al.* and Anderson papers do ***precisely*** this). Yet by now, it

[1] It should be noted that this reference addresses ***non-viral*** delivery systems. Since applicants have elected the species of adenoviral delivery, it seems that an attack on non-viral delivery systems is premature until such time as the election of species requirement is withdrawn.

cannot be argued that gene therapy *per se* is not enabled. The PTO has issued dozens (if not hundreds) of gene therapy patents, and there are hundreds of gene therapy clinical trials going on around the world, including many that have shown clinical benefit (see quote above from Schmidt-Wolf *et al.*). Thus, to start out with the default position of "gene therapy is *per se* not enabled" is factually incorrect. And it is ***not*** sufficient, as a response to this argument, for the PTO to say that "each application is examined on its own merits, and there is no evidence showing efficacy of the ***claimed*** invention" – this is for the simple reason that the PTO is using the alleged deficiencies of ***other*** (and much older) gene therapies to support the present rejection. If the PTO is going to advance ***generalized*** concerns regarding gene therapy, then it must be prepared to consider and address ***generalized*** rebuttals thereof.

C. Specific Concerns Regarding Enablement

The examiner next addresses the timing issue, and it is argued that exposure to the toxin may occur over short or long periods of time, the latter creating problems given the alleged transient nature of gene therapy. In addition, it is argued that levels of toxins, such as in chemical warfare, may overwhelm exogenous PON1 expression, and that information of needed/achieved expression levels is not provided. Applicants submit that it will ***always*** be possible to imagine extreme scenarios where an invention will not work. This is not the proper form of examination for enablement. The question is not whether ***every*** embodiment will work to the maximum potential benefit, but whether there is a reasonable correlation between that which is claimed and that which can be achieved.[1] Here, if there is short-term exposure, then there appears to be little question that vectors can express PON1 short-term, and thus will

facilitate protection. If the exposure is long-term, then at least protection will be afforded in the short-term, and re-administration can be effected to achieve longer-term protection. As for overwhelming PON1 expression with high toxin levels, applicants submit that it is not incumbent upon applicants to establish that in each and every scenario complete protection be provided. In fact, applicants submit that even if toxin levels can outstrip PON1 expression, there would at least have been protection for the subject ***until levels of toxins exceed the protective level of PON1***. This still satisfies the limitation of the present claims, which only requires detoxification, not total elimination of the toxic threat.

D. Mouse Model

Finally, applicants note the examiner's attack on the mouse model described in the examples, and argues that extrapolation to human subjects is not proper. Applicants again disagree with the examiner's reasoning. First, this is not an immunocompromised animal, as is often used in other disease models such as cancer. Second, this is also a situation where the interplay with an animal's endogenous systems, such as the immune system or cardiovascular system, is not required. The simple question is whether one can express a PON1 gene *in vivo* and have it detoxify an organophosphate toxin. The examiner has not offered any concrete reason why protection afforded by PON1 expressed from an adenovirus in a mouse would not provide *prima facie* evidence that the same vector would provide the same expression and protection in a human. Rather, the rejection again reduces to a generic attack on gene therapy that must, at this late stage, be considered outdated.

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[1] Applicants refer the examiner to the amended claims. All that is required is detoxification, which by definition protects the subject from that organophosphate molecule.

The examiner cites a GAO report (but unfortunately does not indicate what portion of the 39-page report is relied upon) for the proposition that extrapolation from animals to humans is unpredictable. Again, applicants point out that this report was three-years old at the time the instant application was filed. Moreover, it does ***not*** provide the basis for denigrating applicants' animal model (no reference to mice could be found anywhere in the GAO report). At most, the report concludes that "the literature does not adequately address ... animal-human extrapolation models ...." Adequately address ***what***? This open-ended statement, made in a context that has ***nothing*** to do with patent law, much less directed at an invention involving adenoviral delivery of PON1 to protect from organophosphates. Thus, it is entirely useless in establishing the enablement or non-enablement of the claimed invention. If anything, this report should be noted for the fact that animal models were reported to show adverse effects from a host of toxins.

At most, the GAO report can be said to criticize the use of research animals to determine whether exposures to low level sarin (a nerve agent), in too low a concentration to produce immediate toxic effects, can cause lasting changes in brain structure or function that further cause chronic ill health. This is a recently raised concern for which there is insufficient research in animal models and in humans to have established mechanisms for disease causation in either the animal models or in humans, much less to know whether findings in animal models can be extrapolated to humans.

But this model is ***not*** the model used by applicants. In contrast, applicants' animal model is based on an entirely different experimental design, namely, whether boosting of PON1 isoenzyme levels in blood protects a mouse from organophosphate toxicity by increasing the rate of destruction of the organophosphate in blood so that it cannot reach brain and other tissues to cause illness. Because this model addresses the destruction of organophosphates in blood ***before***

they get to the brain, it avoids questions of whether and how organophosphates affect tissues once they reach them (the subject addressed by the GAO report).

For three decades it has been established that the main determinant of susceptibility to organophosphate poisoning is the activity level of PON1 isoenzymes, and this relationship has been shown to hold across many species including humans (see of Davies et al. *Nature Genetics* 1996;14:334-336, "interspecies differences in PON1 activity correlate well with observed median lethal dose (LD_{50}) values [of organophophates]" – abstract; citing 3 additional references). It also should be noted that the use of human PON1 genes in the applicants' gene therapy device injected into the mouse model obviated any argument that interspecies variation in PON1 isoenzyme activity clouded the results. Thus, the examiner's objection to extrapolation from applicants' mouse model to humans is directly contradicted by the published literature.

As discussed in the MPEP, an *in vivo* animal model example in the specification, in effect, constitutes a "working example" if that example "correlates" with a disclosed or claimed method. "... [I]f the art is such that a particular model is recognized as correlating to a specific condition, then it should be accepted as correlating unless the examiner has evidence that the model does not correlate. Even with such evidence, the examiner must weigh the evidence for and against correlation and decide whether one skilled in the art would accept the model as reasonably correlating to the condition." MPEP §2164.02. This rule, in essence, directs the examiner here to accept applicants' examples as correlative.

In sum, the evidence of record overwhelmingly supports the enablement of the claimed invention. The arguments made by the examiner have either been rendered moot by the amendments, have been adequately rebutted, are not relevant to the presently claimed invention,

or remain unsubstantiated on the record. As such applicants respectfully request reconsideration and withdrawal of the rejection.

IV. Rejection Under 35 U.S.C. §112, Second Paragraph

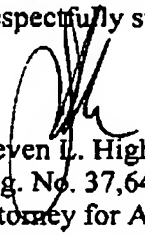
Claims 1, 3-20 and 30 stand rejected under the second paragraph of §112. As discussed above, claim 30 has been canceled, rendering the rejection of this claim moot. Further, claim 1 has been amended to delete "host" from line 3, thereby obviating the rejection. Reconsideration and withdrawal of the rejections is therefore respectfully requested.

V. Conclusion

In light of the foregoing, applicants submit that all claims are in condition for allowance, and an early notification to that effect is earnestly solicited. Should the examiner have any questions regarding this response, a telephone call to the undersigned is invited.

Please date stamp and return the enclosed postcard as evidence of receipt.

Respectfully submitted,



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The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin

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Many organophosphorus compounds (OPs) are potent cholinesterase inhibitors, accounting for their use as insecticides and, unfortunately, also as nerve agents. Each year there are approximately 3 million pesticide poisonings world-wide resulting in 220,00 deaths[1–2]. In 1990, there were 1.36 million kg of chlorpyrifos, 4.87 million kg of diazinon and 1.23 million kg of ethyl parathion manufactured in the USA (data supplied by the USEPA). In addition to exposure risks during pesticide manufacturing, distribution and use, there are risks associated with the major international effort aimed at destroying the arsenals of nerve agents, including soman and sarin. The United States has pledged to destroy approximately 25,000 tons of chemical agents by the end of the decade[3]. The high density lipoprotein (HDL)-associated enzyme paraoxonase (PON1) contributes significantly to the detoxication of several OPs (Fig. 1). The insecticides parathion, chlorpyrifos and diazinon are bioactivated to potent cholinesterase inhibitors[4] by cytochrome P-450 systems[5]. The resulting toxic oxon forms can be hydrolysed by PON1, which also hydrolyses the nerve agents soman and sarin[6] (Fig. 1). PON1 is polymorphic in human populations and different individuals also express widely different levels of this enzyme[7–9]. The Arg_{192} (R_{192}) PON1 isoform hydrolyses paraoxon rapidly, while the Gln_{192} (Q_{192}) isoform hydrolyses paraoxon slowly[8,10]. Both isoforms hydrolyse chlorpyrifos-oxon[8,9] and phenylacetate[6,7,9] at approximately the same rate. The role of PON1 in OP detoxication is physiologically significant[11–15]. Injected PON1 protects against OP poisoning in rodent model systems[12–15] and interspecies differences in PON1 activity correlate well with observed (☰) an lethal dose (LD_{50}) values[8,11,16]. We report herein a simple enzyme analysis that provides a clear resolution of PON1 genotypes and phenotypes allowing for a reasonable assessment of an individual's probable susceptibility or resistance to a given OP, extending earlier studies on this system. We also show that the effect of the PON1 polymorphism is reversed for the hydrolysis of diazoxon, soman and especially sarin, thus changing the view of which PON1 isoform is considered to be protective.

In the course of evaluating the PON1 status of farm workers prior to pesticide exposure during the growing season, we also determined the rates of diazoxon hydrolysis. By plotting the activity distributions for the three substrates, chlorpyrifos oxon, phenylacetate and diazoxon, against the rates of paraoxon hydrolysis, we were able to clearly resolve individuals homozygous for the low-activity paraoxonase isoform (QQ individuals) from heterozygotes (QR individuals) (Fig. 2*a–c*). However, only the plot of diazoxon hydrolysis versus paraoxon hydrolysis (Fig. 2*c*) clearly resolved all three genotypes and at the same time provided important information about the level of enzyme expressed in a given individual. This two-dimensional enzyme analysis provides a complete assessment of an individual's PON1 status (genotype and phenotype). PON1 levels in a given individual are usually very stable over time[17].

One of the most interesting observations was the reversal of the effect of the PON1 activity polymorphism for diazoxon hydrolysis relative to paraoxon hydrolysis (Fig. 2*c*). RR homozygotes (high paraoxonase activity) had lower diazoxonase activities (mean=7948 U/l) than QQ homozygotes (mean=12,318 U/l). Average rates of diazoxon hydrolysis (10,619 U/l) were somewhat higher than the rates of chlorpyrifos oxon hydrolysis (8233 U/l), suggesting that on average, humans may be better able to detoxicate diazinon than chlorpyrifos or parathion.

We also observed an increased frequency for the R_{192} allele (0.41) in this Hispanic population compared with a frequency of 0.31 observed in populations of Northern European origin[18]. This results in approximately 16% of individuals of Hispanic origin being homozygous for the R_{192} PON1 isoform compared with only 9% of individuals of Northern European origin[8,18].

Following the March 1995 release of sarin in the Tokyo subway, we examined the effect of the PON1 polymorphism on soman and sarin hydrolysis, as PON1 is the only enzyme from humans known to hydrolyse the phosphorus-fluorine bond of these very toxic nerve agents[6]. It is clear that the effect of the polymorphism is reversed for both of these compounds, especially sarin (Fig. 2*d*, *e*). The mean value for sarin hydrolysis was only 38 U/l for the R_{192} homozygotes compared with 355 U/l for the Q_{192} homozygotes (Table 1). The ranges of values for hydrolysis of each of the PON1 substrates are also shown in Table 1.

These results help to explain the large individual differences in sensitivity to OP insecticides processed through the P-450/PON1 pathway or hydrolysed directly by PON1. As the dose response curves for OP toxicity are very steep[12], a small percentage difference in metabolic rate can represent a significant difference in OP sensitivity. In this light, it is important to note that we found in earlier studies that newborns have very low levels of PON1[19], leading to the prediction that newborns are probably significantly more sensitive than adults to OP compounds processed by PON1. Increased sensitivity to OPs has been observed in newborn rats[20,21].

In addition to playing a major role in OP detoxication, the PON1 polymorphism has been recently implicated in another important area of human health. Watson *et al.*[22] demonstrated that PON1 destroys biologically oxidized phospholipids, while other investigators have shown that the R_{192} allele represents a risk factor for coronary artery disease[23,24]. These studies suggest that the same considerations given to the determination of both PON1 genotype and phenotype (PON1 status) relative to OP sensitivity will also be important for studies on the role of PON1 in vascular disease.

These studies underline the importance of examining the effects of polymorphisms on each substrate or

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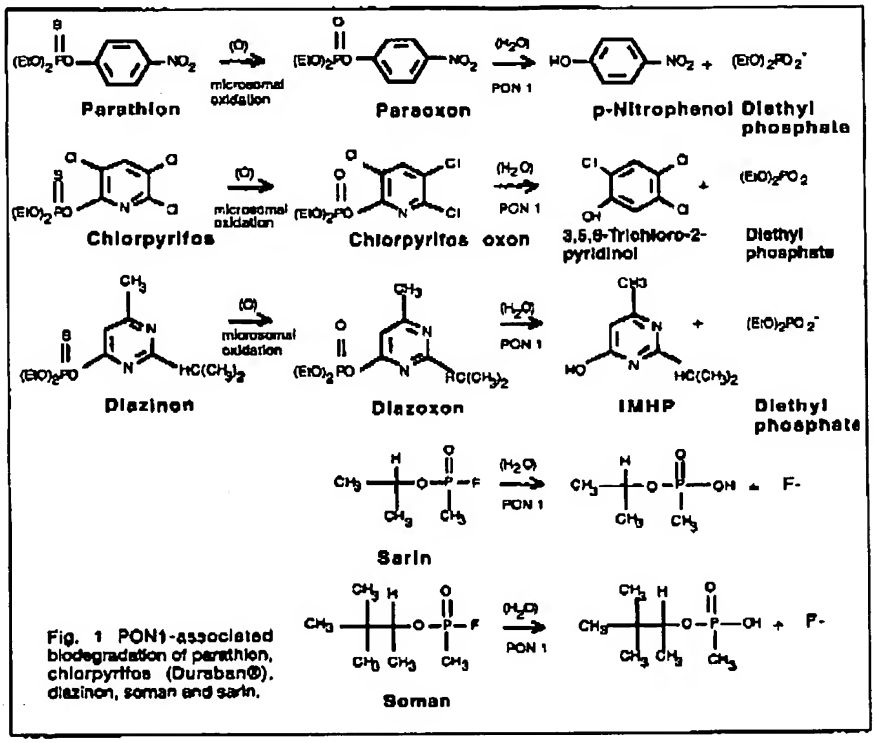

Fig. 1 PON1-associated biodegradation of parathion, chlorpyrifos (Dursban®), diazinon, soman and sarin.

inhibitor of physiological importance. A single amino acid mutation in acetylcholinesterase has been demonstrated to cause a reversal in sensitivities of leaf hoppers to specific OP insecticides[25]. Reversal of sensitivity to inhibitors by single amino acid changes have also been observed in plant[26] and viral[27] systems. The effect of the PON1 polymorphism on sarin hydrolysis illustrates how dramatic the reversal of the effect of an enzyme polymorphism can be.

Methods

Human subjects. Plasma (heparin) from 92 individuals of Hispanic origin were drawn via venipuncture with informed consent.

Enzyme assays. Hydrolysis rates of paraoxon[8], phenylacetate[28] and chlorpyrifos oxon (CPO)[8] were determined as described. Rates of diazoxon hydrolysis were determined by a continuous spectrophotometric assay developed in our laboratory (R.J.R. and C.E.F., manuscript in preparation) based on published

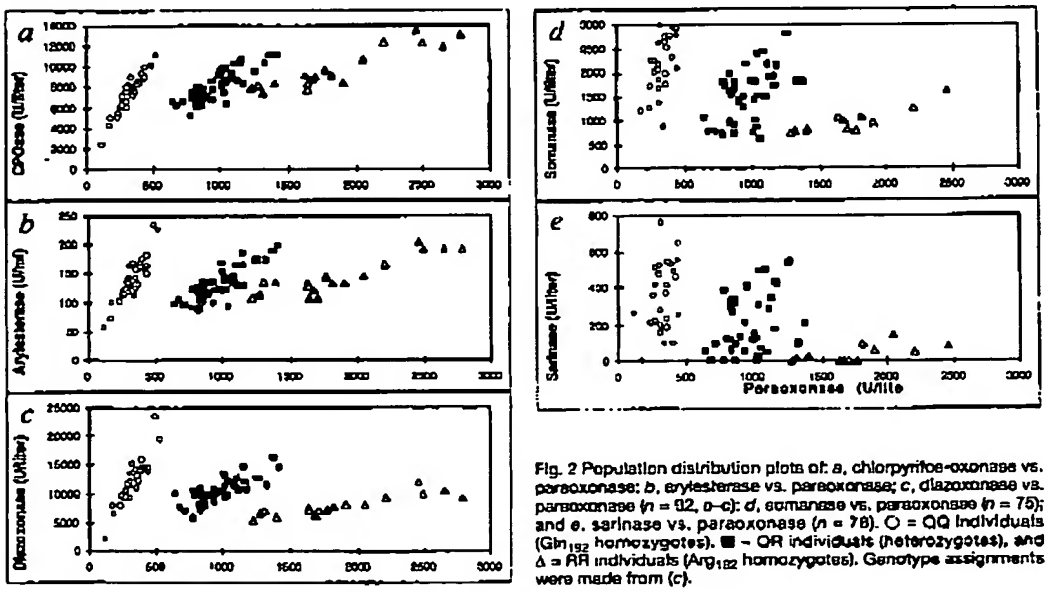

Fig. 2 Population distribution plots of: *a*, chlorpyrifos-oxonase vs. paraoxonase; *b*, arylesterase vs. paraoxonase; *c*, diazoxonase vs. paraoxonase (n = 92, *a*–*c*); *d*, somanase vs. paraoxonase (n = 75); and *e*, sarinase vs. paraoxonase (n = 78). ○ = QQ individuals (Gln_{192} homozygotes). ■ = QR individuals (heterozygotes), and Δ = RR individuals (Arg_{192} homozygotes). Genotype assignments were made from (*c*).

Table 1 Ranges of PON substrate activities in human serum

	Diazoxonase[a] (U/L)		Sarinase[a] (U/L)		Somanase[a] (U/L)	
	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.
All	2174–23316[b]	10619 ± 3207	0–758[c]	230 ± 181	616–2982[d]	1658 ± 660
QQ	2174–23316[e]	12318 ± 3748	0–758[f]	355 ± 183	870–2982[g]	2143 ± 576
QR	5903–1827[h]	10426 ± 2302	0–541[i]	198 ± 161	616–2815[j]	1518 ± 559
RR	5400–11193[k]	7948 ± 1712	0–144[l]	38 ± 47	754–1816[m]	992 ± 263

	Paraoxonase[a] (U/L)		CPOase[a] (U/L)		Arylesterase[a] (U/ml)	
	Range	Mean ± S.D.	Range	Mean ± S.D.	Range	Mean ± S.D.
All	121–2786[b]	924 ± 603	2415–13540[b]	8233 ± 1908	57–235[b]	136 ± 32
QQ	121–532[e]	328 ± 79	2415–11101[e]	7464 ± 1840	57–235[e]	138 ± 37
QR	653–1418[h]	977 ± 171	5134–11160[h]	8152 ± 1519	88–196[h]	131 ± 28
RR	1237–2786[k]	1769 ± 354	7480–13540[k]	9794 ± 2001	106–205[k]	145 ± 32

[a]Assays are described in Methods. [b]n = 92, [c]n = 78, [d]n = 75, [e]n = 33, [f]n = 28, [g]n = 26, [h]n = 41, [i]n = 38, [j]n = 38, [k]n = 16, [l]n = 12, [m]n = 11. All = all individuals in study. QQ = Gln_{191} homozygotes, QR = heterozygotes, RR = Arg_{191} homozygotes.

spectral data[29,30]. The incubation mixtures contained 0.1 M Tris-HCl, pH 8.5, 2.0 M NaCl, 2.0 mM $CaCl_2$, 500 μM diazoxon, and 5 μl of plasma in a volume of 1 ml at 24 °C. Appearance of 2-isopropyl-4-methyl-6-hydroxy pyrimidine (IMHP) was continuously monitored at 270 nm in a Beckman DU-70 spectrophotometer. The reaction was initiated by addition of plasma.

Hydrolysis rates of sarin and soman were determined at the USAMRICD Facility with a titrimetric procedure, using a Radiometer TTT80 pH-stat and an ABU80 autoburette. 3 ml of 1 mM soman or sarin in 1.0 M NaCl with 2.0 mM $CaCl_2$ were added to a temperature-controlled reaction vessel fitted with a capillary delivery tube from the autoburette, and the background hydrolysis rate was monitored for several minutes. Then, 50 μl of plasma were added and the resulting hydrolysis rate monitored at 25 °C. The background rate was subtracted from the sample hydrolysis rate. All samples were measured in triplicate.

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